Enhanced Expression of The *LDH-A* Gene after Gravity-changing Stress in Human RSa Cells

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Abstract A major issue in radiation and space biology is whether gene expression levels are altered in cells exposed to gravity-changing stress. In the present study, genes up- or down-regulated in radiation-sensitive human RSa cells cultured under gravity-changing conditions, were identified using a PCR-based mRNA differential display method. Exposure of cells to gravity-changing stress was performed by free-fall with a drop-shaft facility or by an airplane-conducted parabolic flight. Among the candidates for gravity-changing stress-responsive genes obtained by the differential display analysis, the lactate dehydrogenase A gene (*LDH-A*) was confirmed by Northern blotting analysis to exhibit increased expression levels. The gravity-changing stress consisted of a combination of microgravity and hypergravity. However, exposure of the cells to hypergravity produced by centrifuge only slightly affected the *LDH-A* mRNA expression. Thus, *LDH-A* was found to be a candidate for the genes which play a role in the cellular response to gravity-changing stress, and mainly to microgravity.

Key words; gravity-stress, human cells, gene expression, lactate dehydrogenase A

Introduction

Various biological changes occur in human cells exposed to gravity-changing stress such as space flight and hypergravity produced by centrifuge. For example, in space flight, induction of lymphocyte growth by mitogens was suppressed (Cogoli, 1993; Cogoli *et al.*, 1984), apoptosis was enhanced (Lewis *et al.*, 1998), and production of IL-1 by monocytes was inhibited (Cogoli, 1993). We recently reported an increase in the cell surface expression of CD69 and a decrease of CD23 expression in human lymphocytes without treatment with mitogens after exposure to gravity-changing stress induced by free-fall and parabolic flight (Nomura *et al.*, 2001). There were also reports that space flight causes genetic changes (Testard *et al.*, 1996; Yang *et al.*, 1997).

However, the molecular mechanisms behind the cellular responses to gravity-changing stress are not well established. To clarify these mechanisms it is important to identify those genes whose expression is altered after exposure to gravity-changing stress. In fact, increases in the expression levels of *c-fos* and *c-jun* genes in lymphocytes treated with EGF and TPA were found to be suppressed on exposure of the cells to microgravity in a sounding rocket (de Groot *et al.*, 1990). Furthermore, results of a genetic analysis using microarray technology showed

that expression levels of a number of genes were altered to various extents in human renal cortical cells exposed to gravity-changing stress during space flight (Hammond *et al.*, 1999).

Along with microarray technology, PCR-based mRNA differential display analysis is useful for the detection and characterization of altered gene expression (Liang and Pardee, 1992; Higuchi *et al.*, 1998; Kita *et al.*, 2000a; Kikuno *et al.*, 1998). In addition, human cell lines with high susceptibility to various stress agents may be useful for identifying stress-responsive genes. Actually, we recently analyzed UV-responsive genes in human RSa cells by the differential display method, and found that human Ras homologue enriched in brain (Rheb) is involved in increased UV susceptibility (Kita *et al.*, 2000a). RSa cells were established from human embryonic fibroblast cells doubly infected with Rous sarcoma virus and Simian virus 40, and characterized as a cell line with high sensitivity to UV cell-killing and UV mutagenicity (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981).

In the present study, using the differential display method, we first searched for genes whose expression levels were altered after exposure of RSa cells to gravity-changing stress induced by free-fall in a drop-shaft and by parabolic flight, and next confirmed the alteration by Northern blotting analysis. Expression levels of the lactate dehydrogenase A gene (*LDH-A*) were found to increase early after exposure of the cells to the gravity-changing stress.

Materials and Methods

Cells and culture conditions

RSa cells were established from human embryonic fibroblast cells doubly infected with Rous sarcoma virus

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and Simian virus 40 (Kuwata *et al.*, 1976). Characteristics of the RSa cell line have been described with regard to high sensitivity to far-ultraviolet light (UVC, principally 254 nm in wavelength)-induced cell-killing and mutagenicity (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981). AP^F-1 cells were established as a variant cell line with resistance to the UV mutagenicity as previously described (Isogai *et al.*, 1998; Wang *et al.*, 1999). HeLa cells have been described elsewhere (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981). The medium used for culture was Eagle's minimal essential medium (EMEM) (GIBCO/BRL, Grand Island, NY, USA) containing 10% (v/v) calf serum (CS) (GIBCO/BRL) and antibiotics (100 mg/ml streptomycin and 100 units/ml penicillin G) (Meiji Seika, Tokyo, Japan). Cells were cultured in cell culture flasks with a plug seal cap (Corning, NY, USA) at 37°C in a humidified atmosphere containing 5.0% CO₂.

Exposure to gravity-changing stress

For the free-fall experiment using a capsule in a vacuum shaft, we used drop-shaft facilities at The Micro-Gravity Laboratory of Japan (MGLAB, Toki, Japan) and at Japan Microgravity Center (JAMIC, Kami-sunagawa, Japan), as described elsewhere (Nomura *et al.*, 2001). The facilities of MGLAB and JAMIC allowed for microgravity levels of 10⁻⁵ G for a duration of 4.5 sec or 10 sec, respectively. During a deceleration step after the free-fall, the former capsule produced a maximum of 10 G and the latter a maximum of 8 G for about 0.5 sec.

A Mitsubishi MU-300 aircraft (Mitsubishi Heavy Industries, Tokyo, Japan) operated by Diamond Air Service Co. (Nishikasugai, Japan) was utilized for the parabolic flight experiment as described elsewhere (Nomura *et al.*, 2001). The aircraft ascended to 28,000 feet and then dived to the ground following a parabolic flight path. The aircraft produced a microgravity level of about 3 × 10⁻² G for a duration of 20 sec. It also produced 2 G for 20 sec prior to the parabolic flight and 1.5 G for 20 sec in recovery from the flight. The flight was repeated 10 times. As a control flight, the MU-300 aircraft flew under the same conditions including area, height and duration, except for the parabolic flight.

For the hypergravity experiment, we used the Centrifugal Acceleration Test facility of the Tsukuba Space Center (NASDA, Tsukuba, Japan). Centrifugation of the swing arm produced a maximum force of 10 G for 1 sec or 2 G for 200 sec on the cultured human cells, corresponding to the hypergravity level and duration produced by the free-fall or the parabolic flight, respectively.

Under all of the above gravity-changing conditions, room temperature (about 18–24°C) was maintained. Mock cells were treated in the same manner, except without exposure to gravity-changing stress.

RNA extraction

Total cellular RNA was isolated as follows. Cells were solubilized with TRIZOL (GIBCO/BRL) reagent at the indicated time after exposure to gravity-changing stress induced by free-fall or parabolic flight, and total RNA was

isolated from the cells by guanidine isothiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987) using the TRIZOL reagent. Ten µg of total RNA was freed from contaminating chromosomal DNA with 20 units of RNase-free DNase I (GeneHunter, Nashville, TN, USA).

RT-PCR and differential display

Differential display was performed using a Differential Display Kit (Display Systems Biotech, CA, USA) as described elsewhere (Liang and Pardee, 1992; Kikuno *et al.*, 1998). Briefly, 250 ng of DNase I-treated RNA was used in a reverse transcription reaction with an anchored downstream primer (5'-TTTTTTTTTTTAC-3'). The resulting cDNA was subsequently amplified by PCR using arbitrary upstream primers (E: 5'-AAACTCCGTC-3', K: 5'-GATCTGACAC-3', L: 5'-GATCTAACCG-3', U: 5'-GATCTCAGAC-3', X: 5'-CTTTCTACCC-3') and the downstream primer with 12.5 µCi of [α -³⁵S]dATP (New England Nuclear, Boston, MA, USA). The PCR products were electrophoresed on a 5% denaturing polyacrylamide gel. After electrophoresis, the gel was dried and exposed to Kodak X-Omat X-ray film (Eastman Kodak, Rochester, NY, USA). The cDNA bands that differed between cells with and without (mock) exposure to gravity-changing stress were excised from the dried gel and cDNAs were eluted. The eluted cDNA fragments were reamplified using the same set of primers described above and subsequently cloned into pCR 2.1 vector using TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA).

DNA sequencing

Nucleotide sequences of cloned cDNAs were determined by the dideoxy-mediated chain termination method (Sambrook *et al.*, 1989) using BigDyeTM Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) with the M13 primer according to the supplier's instructions. The resulting products were electrophoresed and analyzed on a ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). Nucleotide sequences of the cDNAs were compared to known sequences in the GenBank/EMBL/DBJ database using the Basic Local Alignment Tool (BLAST) program.

Northern blotting analysis

Northern blotting analysis was performed as described elsewhere (Kita *et al.*, 2000a). Briefly, the cloned cDNAs were excised from the vector and labeled with 100 µCi of [α -³²P]dCTP (New England Nuclear) using BcaBESTTM Labeling Kit (Takara Shuzo Co., Ltd., Kyoto, Japan) and used as probes. The DNase I-treated RNA (10 µg) was electrophoresed on a denaturing agarose gel (1% agarose, 6.7% formaldehyde) and blotted onto Hybond N+ nylon membrane (Amersham Pharmacia, Uppsala, Sweden). The membrane was hybridized with the labeled probes overnight in 5 X SSPE, 5 X Denhardt's solution, 0.5% SDS and 100 µg/ml of salmon sperm DNA at 42°C, washed three times at room temperature in 2 X SSC, 0.1% SDS and then twice at 55°C in 2 X SSC, 0.1% SDS, and autoradiographed. The membrane was stripped and

Fig. 1 Northern blotting analysis of LDH-A mRNA expression levels in RSa cells after exposure to gravity-changing stress induced by free-fall. (A) Total RNA was extracted from cells 1, 8 and 22 h after exposure to free-fall for 10 sec, or mock-treated cells without free-fall exposure (Mock). Northern blotting analysis was performed as described in Materials and Methods. The approximate mRNA size is shown in kb. (B) The amounts of LDH-A mRNA were normalized to those of GAPDH mRNA and shown as a percentage of the value of the mock-treated cells, as described in Materials and Methods. Experiment 1 (●) was derived from the results shown in A, and experiment 2 (○) was based on another independent free-fall experiment.

rehybridized with the probe for the glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) as a control for RNA loading and transfer. Amounts of mRNA were quantified using a BAS-2500 Bioimaging Analyzer (Fuji Film, Tokyo, Japan). Amounts of LDH-A mRNA were divided by those of GAPDH mRNA and the relative expression levels were shown as a percentage of the values of the mock-treated cells without exposure.

Results

Differential display was performed using 5 different combinations of random primers. Seven cDNA bands, which had more than two-fold or less than half the intensity of that of mock-treated cells, were selected in RSa cells exposed to the gravity-changing stress induced by free-fall (Kita *et al.*, 2000b). One of the up-regulated cDNA clones was identical to the human *LDH-A* gene. The change in mRNA expression levels for *LDH-A* was further examined by Northern blotting analysis in RSa cells exposed to gravity-changing stress for 10 sec during free-fall in a drop-shaft. A 1.8-kb transcript was detected (Fig. 1A), and the expression levels increased up to 8 h after exposure, and then decreased (Figs. 1A and 1B). There was no change of the expression levels in mock-treated cells during the time tested (data not shown). Next, effects of the duration of exposure to the gravity-changing stress on the LDH-A mRNA expression were examined. The levels were slightly increased after a 4.5-sec exposure, and greatly increased after a 10-sec exposure (Fig. 2).

The gravity-changing stress induced by parabolic flight

also increased LDH-A mRNA levels in RSa cells (Table 1). The expression was increased 2 h after the parabolic flight and remained high up to 23 h. In the control flight, the levels increased slightly up to at least 9 h after exposure but decreased to the control levels in mock-treated cells

Fig. 2 Effects of the duration of exposure to gravity-changing stress induced by free-fall on the LDH-A mRNA expression levels in RSa cells. Total RNA was extracted from cells 1 h after a 4.5-, 10-sec exposure to free-fall, or from mock-treated cells without free-fall exposure (Mock). Northern blotting was performed and the relative mRNA levels of LDH-A to those of GAPDH are shown as a percentage of the value of the mock-treated cells, as described in Materials and Methods. The 4.5- and 10-sec free-fall experiments were performed three times. Bars indicate the standard deviations.

Table 1 Expression levels of LDH-A mRNA in RSa cells after exposure to gravity-changing stress induced by parabolic flight.

Time (h) after exposure ^a	Expression ^b		Relative inducibility ^c
	PF ^d	CF ^d	
	(% of mock)	(% of mock)	(PF/CF)
2	159	115	1.38
9	141	115	1.23
23	161	86.9	1.85

^a The time indicates the period after the last PF and after the corresponding CF.

^b The relative levels of LDH-A mRNA to those of GAPDH mRNA after PF and CF are shown as a percentage of the value in mock-treated cells without exposure, as described in Materials and Methods.

^c The inducibility of the LDH-A mRNA expression at each time point after PF was divided by that after CF.

^d PF, parabolic flight; CF, control flight.

(Table 1). The relative inducibility of the up-regulated expression by parabolic flight to that by control flight was highest 23 h after exposure (Table 1).

The expression levels of LDH-A mRNA in RSa cells increased only slightly following exposure to hypergravity as compared to conditions of 1G in mock-treated cells: the LDH-A mRNA levels relative to those of GAPDH 1 h after exposure to hypergravity of 2 G for 200 sec and 10 G for 1 sec in the Centrifugal Acceleration Test facility increased by 15% and 19%, respectively.

LDH-A expression levels after exposure to gravity-changing stress in free-fall were further investigated in other established human cell lines. In both AP^F-1 and HeLa cells, the increases of the levels were similar to those in RSa cells (Table 2).

Discussion

Recently, LDH-A gene was reported to be up-regulated in human renal cortical cells exposed to gravity-changing stress in space flight (Hammond *et al.*, 1999). LDH-A mRNA expression levels increased about 2.5-fold in the cells cultured for 6 days on the space flight compared to the cells cultured for the same period on the ground. In the present study, the gene was up-regulated in RSa cells exposed to free-fall in a drop-shaft (Figs. 1 and 2). Such an induction was also achieved by parabolic flight (Table 1), while little change was induced by the control flight (Table 1). These results suggested that the induction was caused by exposure to gravity-changing stress rather than other stresses such as the transport of the cultured cells from our laboratory to the experimental facility.

The present experiments using a drop-shaft and aircraft to induce the gravity-changing stress could not separate the effects of microgravity and hypergravity. The results therefore represented an analysis of gene expression in RSa cells subjected to the combined effect of these forces. The effects of hypergravity on LDH-A mRNA expression were therefore examined by exposing the cells to centrifugation at the Centrifugal Acceleration Test facility of NASDA. Hypergravity only slightly affected the expression levels,

Table 2 Comparison of the expression levels of LDH-A mRNA among RSa, AP^F-1 and HeLa cells after exposure to gravity-changing stress induced by free-fall.

Cell	LDH-A mRNA levels ^a
RSa	173
AP ^F -1	166
HeLa	156

^a The LDH-A mRNA levels relative to those of GAPDH mRNA in cells 1h after exposure to free-fall are shown as a percentage of the value of the respective mock-treated cells without exposure, as described in Materials and Methods.

suggesting that the up-regulation of the LDH-A gene was mainly due to microgravity. However, in the experiment of a 4.5-sec exposure to free-fall, hypergravity possibly contributed much to the slight increase in the gene expression.

LDH catalyzes the interconversion of pyruvate and lactate. In anaerobic cells, conversion of pyruvate to lactate regenerates NAD⁺ and is essential for continued glycolytic flux. The LDH-A gene encodes the M subunit of the enzyme; the isoform that is adapted for anaerobic function. Previous study has established that expression of LDH-A mRNA is inducible by hypoxia in rat skeletal muscle cells (Robin *et al.*, 1984), mouse fibroblasts (Firth *et al.*, 1994) and cultured vascular smooth muscle (VSM) cells derived from rat aorta (Marti *et al.*, 1994), and by cAMP in rat VSM cells (Marti *et al.*, 1994) and rat glioma cells (Miles *et al.*, 1981; Jungmann *et al.*, 1983). The physiological role of the increased expression of the LDH-A gene in RSa cells exposed to gravity-changing stress is yet unclear. However, the increase might be partly related to cellular adaptation to anaerobic conditions, because under microgravity conditions, the absence of convection may prevent the replenishing of metabolic gases and cause anaerobic conditions in the cells (Porterfield *et al.*, 1997). Actually, reduction of glucose consumption was reported in human embryonic lung cells cultured in microgravity conditions (Montgomery *et al.*, 1978).

Interestingly, LDH has been suggested to reside in nuclei of mammalian cells, to bind to single-stranded DNA and to exert functions in DNA replication, transcription and repair (Calissano *et al.*, 1985; Grosse *et al.*, 1986; Xia *et al.*, 1994). Further, Popanda *et al.* reported that LDH stimulated UV-induced DNA repair in human fibroblasts (Popanda *et al.*, 1998). Therefore, another possibility is that the up-regulated LDH-A mRNA contributes to the functions via binding to DNA in nuclei. We here used three cell lines with different sensitivities to UV. RSa cells have high sensitivity to UV-mutagenicity and cell-killing (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981). In contrast, AP^F-1 and HeLa cells are both resistant to UV-induced mutagenicity (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981; Isogai *et al.*, 1998; Wang *et al.*, 1999), and the latter cells were further resistant to UV cell-killing (Kuwata *et al.*, 1976; Suzuki and Fuse, 1981) because of their higher repair

capacity than RSa cells. However, the gravity-changing stress-induced up-regulation of the *LDH-A* gene was observed in all the cell lines, and to a similar extent. These findings suggested that the increased *LDH-A* expression levels were not correlated with the functions involved in the UV susceptibility among the cells tested.

The present results showed that free-fall in a drop-shaft could induce an alteration of the gene expression similar to that induced by space flight and thus the free-fall experiment is useful for analysis of the mechanisms of some responses induced by gravity-changing stress in human cells. We obtained some other cDNA clones as candidates for gravity-changing stress-responsive genes including translocation-associated membrane protein (*TRAM*) and histone acetyl transferase (*HAT*) genes. More detailed analysis of the expression levels of the genes as well as functional analysis of *LDH-A* is now under way.

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References

- Cogoli, A. (1993) The effect of hypogravity and hypergravity on cells of the immune system. *J. Leukocyte Biol.*, **54**, 259-268.
- Cogoli, A., Tschopp, A. and Fuchs-Bislin, P. (1984) Cell sensitivity to gravity. *Science*, **225**, 228-230.
- Lewis, M. L., Reynolds, J. L., Cubano, L. A., Hatton, J. P., Lawless, B. D. and Piepmeier, E. H. (1998) Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). *FASEB J.*, **12**, 1007-1018.
- Nomura, J., Arase, Y., Sugita, T., Sugaya, S., Takahashi, S., Kita, K., Yamamori, H., Sekiya, S., and Suzuki, N. (2001) Increased and decreased expression of CD69 and CD23, respectively, in gravity-stressed lymphocytes. *Avi. Space Env. Med.*, **72**, 727-732.
- Testard, I., Ricoul, M., Hoffschir, F., Flury-Herard, A., Dutrillaux, B., Fedorenko, B., Gerasimenko, V. and Sabatier, L. (1996) Radiation-induced chromosome damage in astronauts' lymphocytes. *Int. J. Radiat. Biol.*, **70**, 403-411.
- Yang, T. C., George, K., Johnson, A. S., Durante, M., and Fedorenko, B. S. (1997) Biodosimetry results from space flight Mir-18. *Radiat. Res.*, **148**, S17-S23.
- de Groot, R.P., Rijken, P.J., den Hertog, J., Boonstra, J., Verkleij, A. J., de Laat, S. W., Kruijer, W. (1990) Microgravity decreases *c-fos* induction and serum response element activity. *J. Cell Sci.*, **97**, 33-38.
- Hammond, T. G., Lewis, F. C., Goodwin, T. J., Linnehan, R. M., Wolf, D. A., Hire, K. P., Campbell, W. C., Benes, E., O'Reilly, K. C., Globus, R. K. and Kaysen, J. H. (1999) Gene expression in space. *Nature Medicine*, **5**, 359.
- Liang, P. and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science*, **257**, 967-971.
- Higuchi, Y., Kita, K., Nakanishi, H., Wang, X. L., Sugaya, S., Tanzawa, H., Yamamori, H., Sugita, K., Yamaura, A. and Suzuki, N. (1998) Search for genes involved in UV-resistance in human cells by mRNA differential display: Increased transcriptional expression of Nucleophosmin and T-Plastin genes in association with the resistance. *Biochem. Biophys. Res. Comm.*, **248**, 597-602.
- Kita, K., Wu, Y. P., Sugaya, S., Moriya, T., Nomura, J., Takahashi, S., Yamamori, H., Nakajima, N. and Suzuki, N. (2000a) Search for UV-responsive genes in human cells by differential mRNA display: Involvement of human Ras-related GTP-binding protein, Rheb, in UV susceptibility. *Biochem. Biophys. Res. Comm.*, **274**, 859-864.
- Kikuno, K., Kita, K., Nomura, J., Hiwasa, T., Yonemitsu, H. and Suzuki, N. (1998) Search for genes responsible for UV susceptibility of human cells: Involvement of Syndecan-1 in UV resistance. *Biochem. Biophys. Res. Comm.*, **253**, 519-523.
- Kuwata, T., Oda, T., Sekiya, S. and Morinaga, N. (1976) Characteristics of a human cell line successively transformed by Rous Sarcoma virus and Simian virus 40. *J. Natl. Cancer Inst.*, **56**, 919-926.
- Suzuki, N. and Fuse, A. (1981) A UV-sensitive human clonal cell line, RSa, which has low repair activity. *Mutat. Res.*, **84**, 133-145.
- Isogai, E., Ishijima, S., Sonoda, T., Kita, K., Suzuki, H., Hasegawa, R., Yamamori, H., Takakubo, Y. and Suzuki, N. (1998) Protease activation following UV irradiation is linked to hypomutability in human cells selected for resistance to combination of UV and antipain. *Mutat. Res.*, **403**, 215-222.
- Wang, X. L., Kita, K., Takahashi, S., Hasegawa, R., Chi, X. J., Yamamori, H., Nakajima, N., Yokoe, H., Tanzawa, H. and Suzuki, N. (1999) Protease activity induced by nicotine in human cells. *Int. J. Mol. Med.*, **4**, 537-540.
- Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156-159.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Kita, K., Moriya, T., Wu, Y. P., Sugaya, S., Takahashi, S., Nomura, J. and Suzuki, N. (2000b) Search for gravity-responsive genes by PCR-based mRNA differential display in human cells. *J. Gravitat. Physiol.*, **7**, 75-76.
- Robin, E. D., Murphy, B. J., Theodore, J. (1984) Coordinate regulation of glycolysis by hypoxia in mammalian cells. *J. Cell Physiol.*, **118**, 287-290.
- Firth, J. D., Ebert, B. L., Pugh, C. W. and Ratcliffe, P. J. (1994) Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: Similarities with the erythropoietin 3' enhancer. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 6496-6500.
- Marti, H. H., Jung, H. H., Pfeilschifter, J. and Bauer, C. (1994) Hypoxia and cobalt stimulate lactate dehydrogenase (LDH) activity in vascular smooth muscle cells. *Eur. J. Physiol.*, **429**, 216-222.
- Miles, M. F., Hung, P. and Jungmann, R. A. (1981) Cyclic AMP

- regulation of lactate dehydrogenase: quantitation of lactate dehydrogenase M-subunit messenger RNA in isoproterenol- and N6, O2'-dibutyryl cyclic AMP-stimulated rat C6 glioma cells by hybridization analysis using a cloned cDNA probe. *J. Biol. Chem.*, **256**, 12545-12552.
- Jungmann, R. A., Kelley, D. C., Miles, M. F. and Milkowski, D. M. (1983) Cyclic AMP regulation of lactate dehydrogenase. Isoproterenol and N6, O2'-dibutyryl cyclic amp increase the rate of transcription and change the stability of lactate dehydrogenase a subunit messenger RNA in rat C6 glioma cells. *J. Biol. Chem.*, **258**, 5312-5318.
- Porterfield, D. M., Crispi, M. L. and Musgrave M.E. (1997) Changes in soluble sugar, starch and alcohol dehydrogenase in *Arabidopsis thaliana* exposed to N₂ diluted atmospheres. *Plant and Cell Physiol.*, **38**, 1354-1358.
- Montgomery, P. O'B., Cook, J.E., Reynolds, R. C., Paul, J. S., Hayflick, L., Stock, D., Schulz, W. W., Kimsey, S., Thirolf, R. G., Rogers, T. and Campbell, D. (1978) The response of single human cells to zero gravity. *In Vitro*, **14**, 165-173.
- Calissano, P., Volontè, C., Biocca, S. and Cattaneo, A. (1985) Synthesis and content of a DNA-binding protein with lactic dehydrogenase activity are reduced by nerve growth factor in the neoplastic cell line PC12. *Exp. Cell Res.*, **161**, 117-129.
- Grosse, F., Nasheuer, H. P., Scholtissek, S. and Schomburg, U. (1986) Lactate dehydrogenase and glyceraldehyde-phosphate dehydrogenase are single-stranded DNA-binding proteins that affect the DNA-polymerase- α -primase complex. *Eur. J. Biochem.*, **160**, 459-467.
- Xia, X., Werner, D., Popanda, O. and Thielmann, H. W. (1994) Expression of mitochondrial genes and DNA-repair-related nuclear genes is altered in xeroderma pigmentosum fibroblasts. *J. Cancer Res. Clin. Oncol.*, **120**, 454-464.
- Popanda, O., Fox, G. and Thielmann, H. W. (1998) Modulation of DNA polymerases α , δ and ϵ by lactate dehydrogenase and 3-phosphoglycerate kinase. *Biochim. Biophys. Acta*, **1397**, 102-117.